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ANTIOXIDANT ACTIVITIES OF PHALERIA MACROCARPA FRUITS EXTRACTED BY AQUEOUS EXTRACTION METHOD

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ABSTRACT

Alcohol and chloroform were common solvents used for extraction, and might cause harmful environmental effect if not handle the disposal properly. This study was carried out to determine the antioxidants activity if *P.macrocarpa* fruit which extracted by aqueous solvent. The extraction was carried out using Soxhlet extraction method. Crude extracted were then measured its antioxidants activities and capacities, by using three different assay, consists of Free Radical Scavenging Activity and its half maximal inhibitory concentration, Ferric Reducing Power Assay, and Total Phenolic Content. The results obtained from the analysis were 80.114% scavenging activity, 92.95% of half maximal inhibitory at concentration of 62.5 mg/mL, 5199.02 µmol Fe^{2+}/100 mL for reducing power of ferric, and 369.1 mg GAE/g for total phenolic content. Since water is polar solvent, it gives significant results of antioxidants activities from extracted

sample. It showed that the presence of phytochemicals from *P.macrocarpa* extract was significantly high and the aqueous solvent used does not lowered the antioxidants readings.

INTRODUCTION

Phaleria macrocarpa (Scheff.) Boerl, is one of the plant from Thymelaeceae family. This species known to be many names such as 'Crown of God', 'Mahkota Dewa' and 'Pau'. This plant grows up to 5-18 m tall, which consists of green and acuminate leaves with length and diameter of 7-10 cm and 3-5 cm respectively. This plant has white flowers and fruits which are green in color when unripe and turn to a vibrant red once it ripened. The seed is round, white and it is not advisable to be consumed directly since it is poisonous [1]. Fruits of P.macrocarpa are said to be a positive medicinal effects towards hypertension, gout, dermatology sickness, liver illness, cancer and diabetes. The stalks have been used to treat bone cancer, its pericarp used as a remedy for breast cancer, cervix cancer, allergies, blood illness and tumors [2].

Past records showed P.macrocarpa's fruit and barks contained saponins, alkaloids, poly phenolics, phenols, and lignins. Due to existence of high secondary metabolites, it is reported that this species has remedial activities and has been used as anti-tumor, anti-hyperglycemia, anti-inflammation, anti-oxidant, and gave vasodilator effects. Studies also show that phenolic compounds and flavonoids in plant may act as reducing agent either by donating hydrogen atom or by reducing singlet oxygen which elucidate their antioxidant activities. P.macrocarpa fruit extract once subjected to reversed-phase high performance chromatography (RP-HPLC), shows flavonoids compounds such as kaempferol, myricetin, naringin, and rutin were found in the extract. These compounds were responsible in positive effect towards alpha-glucosidase activities [3, 4].

Antioxidants defined as a substance which at only low concentration, it will give significantly prevention of oxidation from easily oxidized substrates. Oxidation is an important reaction for every living thing which by this reaction can produces free radicals [5]. Phaleria macrocarpa tree contain a wide range compounds which may provide a traditional remedies to treat various chronic diseases. Phytochemicals presence in these plants, also known as secondary metabolites, and these secondary metabolites included tannins, terpenoids, alkaloids, flavonoids, mangiferin and others. Mangiferin can be found in P.macrocarpa fruit which give beneficial activity towards inhibition cancerous activities [6].

There were various studies on antioxidants on P.macrocarpa's fruit. However, it was said to be rarely found aqueous solvent extraction procedures used although water is the most polar solvent compared to others. Water considered to be harmless to environment during disposal or residue from extraction process. Polyphenols are naturally polar and soluble in water. Hence, using water as a solvent gives higher extraction yield of polyphenols. Current study was conducted to evaluate antioxidants activities in fruit's extract of P.macrocarpa (Scheff.) Boerl. Several study was done by many researchers on antioxidant by

using reducing oxygen assay, but none of them studied using colorimetric assay. Hence, in this study, P.macrocarpa fruit extract were measured its antioxidant activity using colorimetric assay.

MATERIALS AND METHODS

Sample preparation

Fresh ripe P.macrocarpa (PM) fruits with vibrant red skin color were collected from local farm in Bachok, Kelantan, Malaysia. Collected fruits were washed thoroughly with tap water. The fruits were cut thinly and been dried in the oven for 1 week at 60°C. Dried P.macrocarpa then been grinded using commercial grinder to fiber. Fibrous fruits sample were then extracted by Soxhlet extraction method using distilled water as solvent. Extraction was carried out for 6 hours and the crude extract was subjected to spray-dried using NIRO Spray Dryer for further analysis.

Free radical scavenging activity, DPPH

1,1 Diphenyl-1-picrylhydrazyl (DPPH) determination method was adapted from [7] with some modification. 50 µL crude extract (10 mg/mL) was mixed with 150 µL of DPPH in ethanol (50 mg/mL). The mixture was then incubated in dark at room temperature. The absorbance of radical scavenging activity was measured using Microplate Reader 96-well at wavelength 517 nm. Ascorbic acid (AA) was used as positive control while ethanol mixed with DPPH as negative control. Percentage of scavenging inhibition was expressed by using Equation (1).

$$\% \text{ inhibition} = \frac{Ao - Ai}{Ao} \times 100 \qquad (1)$$

A_o = Absorbance control
A_i = Absorbance sample

Lethal dose (IC_{50}) of free radical scavenging activity was measured using method described by [8] with minor modification. Series of dilution of standard (ascorbic acid) and extract were prepared starting from 1000, 250, 62.5, 31.25, 15.625, and 7.81 ppm. These dilution were dispensed 50 µL into 150 µL DPPH. DPPH mixed with distilled water act as a blank. After incubated for 30 minutes in the dark at room temperature, the absorbance was measured with the same wavelength. Capability to scavenge the DPPH radical was calculated using Equation (2).

$$I = \frac{Ao - As}{Ao} \times 100 \qquad (2)$$

I = inhibition percentage
A_o = absorbance blank
A_s = absorbance sample
Percentage of inhibition was compared to percentage of positive control.

Ferric Ion Reducing Power Assay, FRAP

FRAP method was adopted according to [9] with some modification. Acetate buffer was prepared by mixing 3.1 g of sodium acetate trihydrate with 16 mL acetic acid and marked up to 1 L of distilled water. Approximately weight 0.031 g of 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) was prepared in 10 mL 40 mM HCl. Powdered ferric chloride ($FeCl_3.H_2O$) was weighed approximately 0.054 g and was mixed with 10 mL distilled water. FRAP reagent was prepared by mixing 100 mL acetate buffer, 10 mL TPTZ and 10 mL $FeCl_3$. Analysis was carried out by mixing 100 µL of extract with 3 mL of FRAP reagent and read at 593 nm. Lemon powder (LP) was used for comparison purposes and the amount of mixture as same as sample. Iron sulphate (Fe_2SO_4) was used as positive control. Result obtained was recorded as µmol Fe^{2+}/100 mL extract.

Total phenolic content, TPC

Phenolic content determination in P.macrocarpa extract was adapted from [10] with minor modification. Series of dilution Gallic acid were prepared from 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9 mg/mL. Reaction mixtures included 10 mg/mL extract and gallic acid various concentrations, then been mixed with 500 µL ethanol, 2.5 mL 10% Follin-Coicaltue reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. The mixtures were then been incubated for 2 hour in dark and the absorbance were measured at 765 nm. In this analysis, gallic acid was used as positive control. Concentration of phenolics (mg/mL) was obtained by plotting standard calibration curve. The phenolic content in the extract was expressed in terms of gallic acid equivalent (mg gallic acid/g extract).

RESULTS AND DISCUSSION

Summary for overall antioxidants obtained were recorded in the Table 1 provided at the end of the discussion

Free radical scavenging activity on P.macrocarpa fruit

Definition of antioxidant in when a substance significantly able to delays or inhibits the oxidation process. This analysis was determined its inhibition rate of oxidation process in the presence of antioxidant in the extract. DPPH is a stable organic radical which exist in crystalline form, as well as in solution, and it is widely used to determine the anti-radical activity of the compound or natural products' extract. The anti-oxidant activity of a compound or extract also frequently related to radical-scavenging activity [11 – 13]. Results obtained from DPPH assay was 80.114% at concentration of 10 mg/mL. The scavenging activity of P.macrocarpa fruit considered high since the scavenging activity on ascorbic acid was 81.784%. Comparing these two results using Tukey's test, p value obtained was (p=0.830) which considered as have significant different between standard ascorbic acid and P.macrocarpa extract. Previous study showed anti-radical activity from extraction process using ethyl chloride were 71.91%, which slightly lower than extraction using other solvent [10]. Based on

readings, it shows that aqueous extract have the capability in scavenging radical higher than ethyl chloride extract due to polarity of the solvent. General definition of IC_{50} is lethal dose which is an indication the toxicity for given substances or type of radiation. The resistance of radicals varies from individuals due to certain concentration, by calculating the subject will denatured by 50% of the population. Lethal dose of P.macrocarpa extract was determined by 50% scavenging activity occurred between the extract and DPPH assay. Table 1 showed the scavenging activity of the extract increased gradually until it reached certain concentration, which at 62.5 mg/mL. At this concentration, the scavenging activity seems to be stagnant until concentration 1000 mg/mL. By this illustration, it can be concluded that at concentration of 62.5 mg/mL, extract of P.macrocarpa inhibit half of the scavenging assay and gave the percentage of scavenging activity of 92.95%. Referring to Figure 2 given, P.macrocarpa extract inhibit higher compared to ascorbic acid although at the lowest concentration. By this finding, it can be concluding that P.macrocarpa have the ability to scavenging the radical comparable to ascorbic acid.

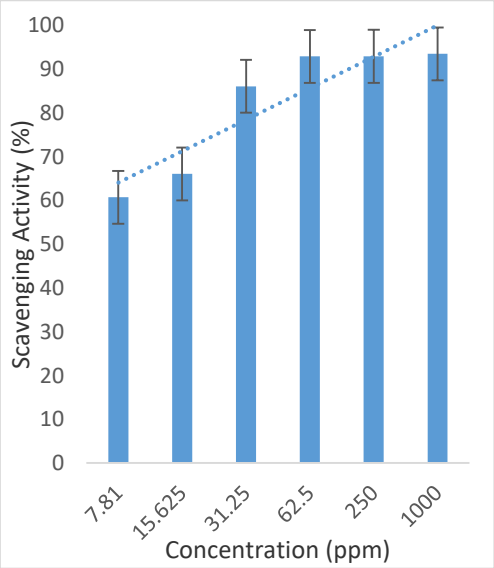

Figure 1: Percentage scavenging activity lethal dose (IC_{50}) of P.macrocarpa extract.

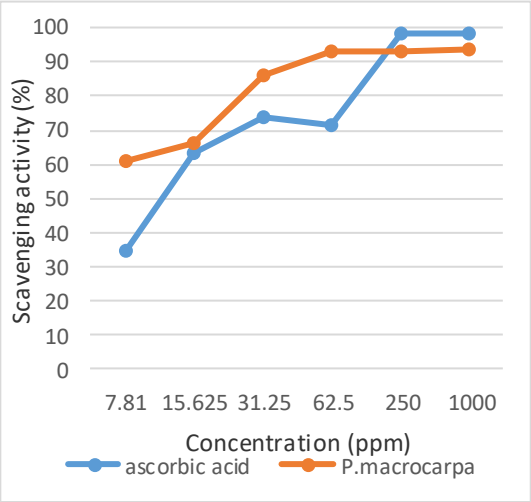

Figure 2: Percentage Free Radical Scavenging Activity Ascorbic acid and P.macrocarpa extract.

Ferric ion reducing power assay

FRAP assay was used in determine the antioxidant capacity of the extract. Mechanism of the reaction occur when the extract reduced ferric ion to ferrous ion, as a result of the reaction, blue-colored ferrous trip yridytrizaine complex (Fe^{2+}-TPTZ) at pH 3.6 formed [14]. Ferrous sulphate solution was used as a standard calibration curve. Standard curve obtained was y= 2.2134x + 0.4711 (R^2 = 0.9952). In this analysis, lemon powder was used as positive control. Data obtained for both extract and lemon powder was 2.58332 and 1.987 respectively. Concentration of extract to reduced ferric ion in TPTZ was 5199.02 µmol Fe^{2+}/100 mL while lemon powder was 48690 µmol Fe^{2+}/100 mL. Based on calculation, there were significant difference between the extract and lemon powder with p value 0.001 ($p<0.005$). This shows that P.macrocarpa extract have the capacity in reducing metal ion greater than lemon powder. According to Xi and team, lemon flesh gives the lowest FRAP values compared to its peels and seeds. Hence, it might give the effect of low ferric reducing power towards lemon powder [15]. According to Nadri et al. (2014) FRAP assay and DPPH were related to each other. If the capacity of scavenging is high, the reducing power will high as well [16].

Phenolic content in P.macrocarpa Fruit

The antioxidant activity happened when the phenols from the extract loses it H^+ ions and produced phenolate ions which had been reduced by follin-Coicalteu reagent. This can be seen through the observation where the color of the assay changes from yellow to blue. The measured of TPC can only be done by comparing with other polyphenols compound as a calibration curve. Total Phenolic Content in P.macrocarpa extract was computed by standard calibration curve of y = 0.0011x + 0.0734, R^2 = 0.9929. Concentration of phenolic in the

extract for 10 mg/mL was calculated using the equation and the absorbance obtained. As the result, phenolic contained in the extract was 369.1 ± 36.8 mg GAE/ g extract with p value 0.569 (p<5) using Tukey's test while phenolic content for ascorbic acid was 413.1 ± 118.6 mg GAE/ g extract with p value 0.435 (P<5). In Figure 3, phenolic content of the extract lies on concentration of gallic acid ranging between 250 – 500 ppm. It shows that P.macrocarpa had significantly high phenolic content although at 10 ppm of the crude extract. There were previous study done on microwave-and ultrasonic-assisted extraction process for P.macrocarpa using ethanolic solvent. The result showed that the TPC were lower at 62.25 ± 0.01 mg GAE/g powder [17].

According to Nadri et al. (2014), phenolic extract using chloroform, gave low phenolic reading, 74.39 mg GAE/g sample. On the other hand, a study done on ethyl acetate, which gave the reading higher compared to chloroform (145.26 mg GAE/g sample) [16]. Apart from that, Hendra et al. (2011) had done using methanolic extract, resulted phenolic content of 60.5 mg GAE/g sample [10]. Comparing to these five solvents on extraction process from ethanol, chloroform, ethyl acetate, methanol, and water, it shows that aqueous extraction gave the highest phenolic content from P.macrocarpa fruit extract. This shows that different solvent used, gave a different TPC reading in natural material. A fact that phenol compounds are naturally exists in polar condition which contained phenolic hydroxyl groups, by applying the principle of polarity of solvent, it clearly shows that water is the most polar solvents compared to other four solvents [18].

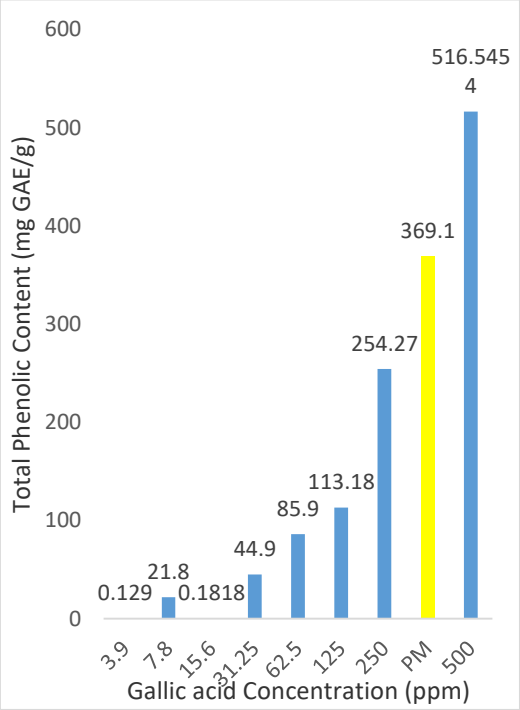

Figure 3: Phenolic Content from various concentration of gallic acid and TPC content from P.macrocarpa extract.

Table 1: Summary of Antioxidants activity on *P.macrocarpa* fruit extract, ascorbic acid, and lemon powder.

Sample	DPPH (%)	FRAP (µmol Fe^{2+}/100 mL extract)	TPC (mg GAE/g)
PM	80.114 **IC_{50}:** 92.95 (62.5 ppm)	5199.02	369.1
AA	81.784	-	413.1
LP	-	48690	-

CONCLUSION

As conclusion, it can be confirmed that aqueous extraction of *P.macrocarpa*'s fruit possesses high significant values of antioxidant activities and capacities. It showed that there were sturdy relation between phenolics and antioxidant activities in water extract's fruits. *Phaleria macrocarpa*'s fruits exhibit a significant source of antioxidants, which makes it as treasured crops. The extraction process might change the view of traditional medicinal remedies for future in pharmaceutical and nutraceutical industries in the future.

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